MONITORING PLAN FOR HAB DEVELOPMENT

1. Objectives

In order to comply with Special Condition 24(b) of EPA Permit No. OD 03-01 the monitoring plan objectives are to (1) monitor the development of any Harmful Algal Blooms (HAB); (2) determine the relationship between any HAB and the dispersal activities; and (3) provide information on whether modification of the dispersal routes and/or timing need to be considered. To accomplish these objectives, a HAB monitoring plan as described below will be implemented.

2. Monitoring/Sampling Methodologies Overview

Data collected during the HAB monitoring will be used to determine the existence of any HAB development, its relationship to the dispersal activities, and any implications for subsequent dispersal tracks and/or timing. Measurements of chlorophyll and nutrients (nitrate, nitrite, ammonia, phosphate, and silicate), as well as hydrographic measurements (temperature, salinity, density as sigma-t units) and satellite observations of water mass movements will continue as the dispersal authorized under the permit takes place. Vessel based HAB monitoring will be conducted using an *in situ* sensor package deployed from a monitoring vessel (probably the Florida Institute of Oceanography (FIO) research vessel (RV) *Bellows* and/or *Suncoaster*, and/or the EPA Ocean Survey Vessel (OSV) *Andersen).* The suite of sensors will provide *in-situ* measurements of salinity, temperature, density (calculated), turbidity, and chlorophyll fluorescence.

3. HAB Monitoring

HAB monitoring will include three components 1) vessel based monitoring activities, 2) satellite imagery review and interpretation, and 3) meteorological/oceanographic data review and interpretation. Over the course of the monitoring, both *in situ* and discrete samples will be collected.

3.1 Vessel Based Monitoring

Objectives:

1) To record background levels of HAB species, nutrients and physical parameters.

2) To record levels of HAB species, nutrients and physical parameters in water masses influenced by the discharge.

3) To record levels of HAB species, nutrients and physical parameters within any suspicious (anomalous) water features identified through the satellite monitoring in order to determine if a HAB has developed.

Prior to each cruise, FDEP will consult with colleagues responsible for the satellite monitoring and determine the location of our Argos tracked drifters to determine the cruise track for each transect. Because of the limitations of ship availability there may be insufficient time to expand the areal coverage of the dispersal area. Therefore, FDEP will co-ordinate our sampling program with the dispersal track of the barge and the most probable direction of the current based on the history of satellite pictures and drifter movements. Our 10 day time frame for monitoring is based on vessel availability and, at least initially, on the typical growth rates of phytoplankton in these oligotrophic waters. Our cruise track includes stations that are located outside of the disposal area. These locations will provide conditions in an adjacent area that can be used for comparison with conditions within the discharge zone. While this may not constitute a statistically discrete control location, it is the best that can be done given the resources available, the areal extent of the discharge zone, and the lack of prior, long term time series of physical, chemical and biological measurements for this region of the eastern Gulf of Mexico.

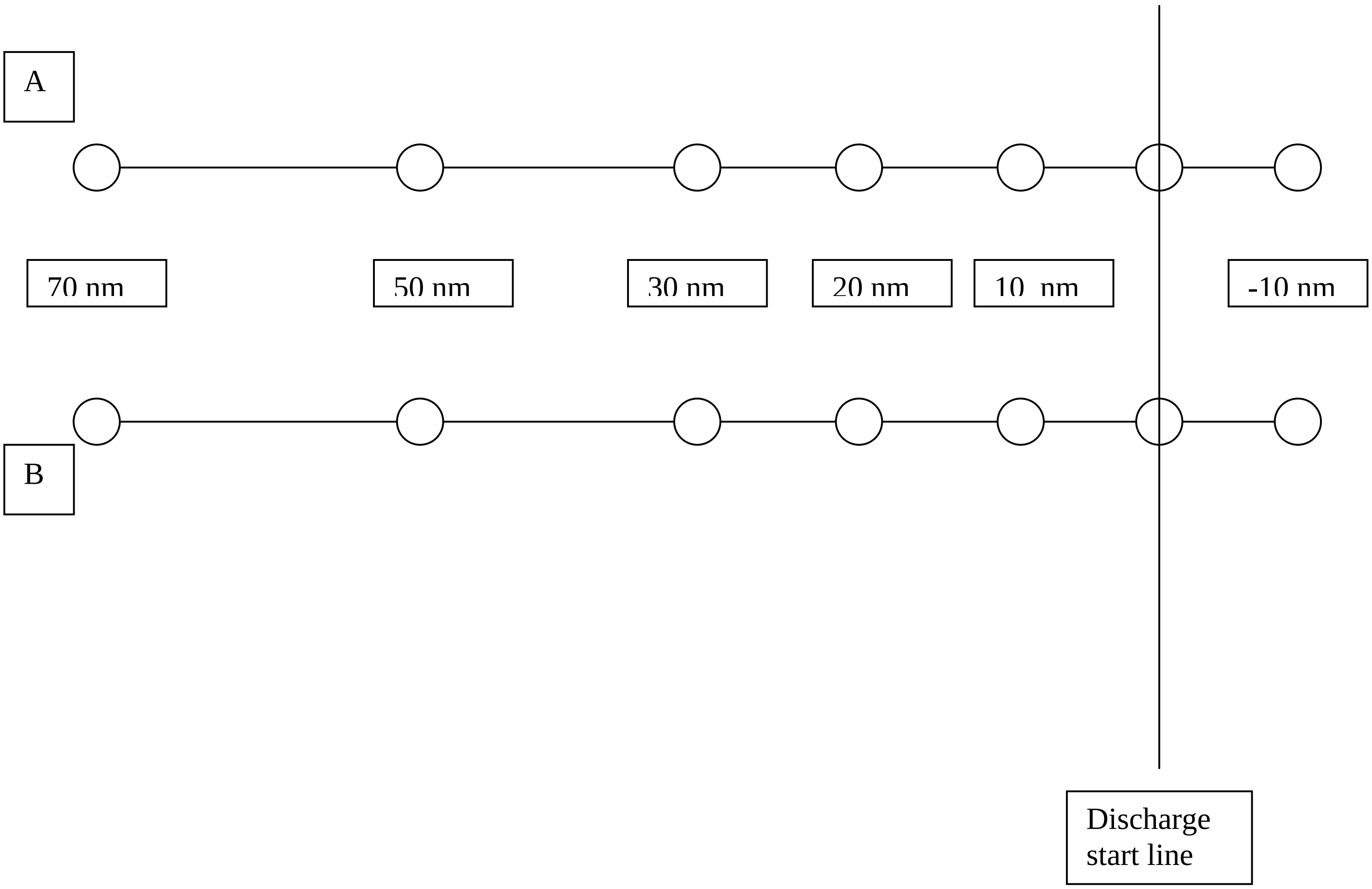
Based upon survey vessel availability, an attempt will be made to perform a background survey prior to the initiation of discharge. Approximately ten days after the first discharge, and 10 days after each subsequent discharge for the first month, repeat monitoring surveys will be attempted (depending on vessel availability). The initial transect line will be along the dispersal vessel track, and the second transect line will be placed at an appropriate distance in a down-current direction of the dispersal line (based upon review of satellite imagery and other meteorological/oceanographic information as described in this plan). Using information gathered during these monitoring events (and vessel availability), a determination will be made for repeat frequency of subsequent monitoring events.

Existing availability of monitoring vessels from FIO (*RV*'s *Bellows* and *Suncoaster*) are as follows:

August 18 - 20	(*RV Suncoaster*)
August 27 - 29	(*RV Suncoaster*)
September 3-5	(*RV Suncoaster*)
September 22 - 25	(*RV Bellows*)
October 1 – 3	(*RV Suncoaster*)
October 15 – 17	(*RV Suncoaster*)
November 12 – 14	(*RV Suncoaster*)
November 24 – 26	(*RV Suncoaster*)
December 14 – 16	(*RV Suncoaster*)

Two parallel transects will be monitored for HAB development. Continuous recording through a flow-through system will be made with a CTD and fluorometer along these transects. Seven stations along each transect line (as depicted in Figure 1) will be visited for vertical CTD profiles from the surface to 50 m depth. Based upon fluorescence maxima and any low surface salinity readings obtained, grab samples will be collected at each location within the mixed layer for HAB species (cell counts and taxonomy by FMRI; samples will be delivered both live and preserved with Lugol's) and chlorophyll-a following 100% methanol extraction (Holm-Hansen and Reimann, 1979). Duplicate samples for chlorophyll-a will be taken at the surface and other depths (maximum of 3), which will be determined by the fluorescence profile. In addition, surface samples will be collected at all stations for the following nutrient analyses: PO_4, SiO_2, NH_4, and NO_3 + NO_2. Analyses for the chlorophyll and nutrient samples will be performed by USF.

Figure 1. HAB development monitoring survey lines (nm = nautical miles).



3.2 Satellite Monitoring

Objective: To observe chlorophyll levels, ocean color, temperature, and sea surface height in order to monitor the movement of water masses in the dispersal area and to identify any suspicious (anomalous) water features for further vessel investigations.

The University of South Florida College of Marine Science (USF/CMS) Remote Sensing Facility available for this project includes a High Resolution Picture Transmission (HRPT) ground station and a new X-band earth station with real-time, high-resolution data capture capability for the SeaWiFS, NOAA Polar Orbiters, and NASA's Terra and Aqua satellites (for MODIS). MODIS direct broadcast (DB) data are captured and processed in near real-time, and archived at USF/CMS (http://modis.marine.usf.edu). The facility also includes computer equipment (12 SGI Octane and O2 workstations and numerous high-end PCs) and software that allows direct implementation of the GSFC/Univ. Miami SeaWiFS/CZCS database software, SEADAS, DSP, and U. Miami MODIS processing software. For data archiving, there are two 600-CD jukeboxes, one 600-DVD jukebox, and data (hard disk) servers with greater than 1000 GB space. The Remote Sensing Facility maintains current licenses for IDL and ENVI (RSI), Erdas Imagine, and Arc-Info. More information can be obtained at http://www.marine.usf.edu and http://imars.marine.usf.edu.

In order to assist in evaluating HAB development, the Institute for Marine Remote Sensing (IMaRS) at USF/CMS will monitor water color and temperature with satellite remote sensing. Specifically, IMaRS will generate high-resolution daily (250-m) maps for the dispersal area and put them online for web broadcasting. Water color is related to the concentrations of various water constituents in both particulate and dissolved forms, namely phytoplankton (through its photosynthetic pigment, chlorophyll-*a*) and its associated debris, dissolved matter, and suspended particles. Water clarity can be derived from water color as well. These parameters, as well as the temperature, can be estimated from satellite measurement and can be used to infer the biological and chemical state of the ocean. In particular, for this project we are trying to identify any suspicious water features (compared with historical data for the same discharge location as well as with concurrent data from other locations) and to trace their movement.) IMaRS personnel may write code to generate tailored product maps (customize the data processing to obtain better image quality and quantitative estimates of water constituents) and will visually examine and interpret the images daily. These images generated by IMaRS using satellite data captured by IMaRS from the MODIS (for chlorophyll and color) and SeaWiFS instruments (for chlorophyll and color data), and AVHRR instrument (for sea surface temperature). IMaRS will monitor the movement of different color features by checking consecutive (within hours to days) images. IMaRS will also assess HAB development (based on a color classification scheme developed at ImaRS; HABs typically have low signal in all spectral bands, different from other types of features) in the dispersal area and the nearby ocean by analyzing real-time and historical (IMaRS has captured and archived temperature data from 1994, and color data from 1997) remote sensing data, and will submit to FDEP a final report with detailed analysis to document any anomalies found associated with the discharge.

3.2.1 Satellite Monitoring Deliverables

IMaRS/CMS/USF will generate two types of deliverables.

1) Online satellite imagery through http://imars.usf.edu.
These images will be tailored color images and data products from SeaWiFS, MODIS, and AVHRR sensors. They will include, but are not limited to:

Color images (true-color composite) from SeaWiFS and MODIS to visualize any possible color change due to the discharge. Images from SeaWiFS are about 1-km resolution, while images from MODIS are about 250-m resolution.

Image data products, including surface chlorophyll concentration, dissolved matter concentration (using absorption as proxy), water clarity index (using attenuation as proxy), particulate concentration (using backscattering as proxy), and surface temperature, will be generated and made available through online access.

These images and data products will be generated and made available online in near real-time to facilitate timely analysis by IMaRS research personnel and by FDEP. This is for fast response to any anomaly event – once an anomaly event is identified, FDEP and EPA will be notified for field sampling in a timely fashion; note that SeaWiFS images and data are properties of Orbimage and therefore are password protected. Authorization from Orbimage is required to be able to access SeaWiFS data (IMaRS generates and archives these data – but they belong to Orbimage. They can be used for non-commercial purpose, but they can’t be distributed freely to any non-authorized user).

2) Two technical reports will be generated. A mid-term report (after two months of the project start) will be prepared and submitted to EPA to document the preliminary findings of this project in regard to the discharge. Upon completion of the project, a final report will be prepared and submitted to EPA to document all detailed findings and analysis including comparison with data from similar periods during the past years. Two reports, one mid-term (after two months of the dispersal commencement, and one final (upon completion of the dispersal). These reports will be sent to the agency in hard copy and will also be put online.)

The objective is to see if there is any color and/or temperature anomaly associated with this dispersal. If yes, what is it and how does it evolve. All these remote sensed data are used for this purpose.

3.3 Meteorological/Oceanographic Monitoring

Objective: To observe meteorological/oceanographic conditions in order to predict transport of the discharge plume and to better manage dispersal activities.

USF also operates a real-time Coastal Ocean Monitoring and Prediction System (COMPS) for West Florida. COMPS consists of an array of instrumentation both along the coast and offshore, combined with numerical circulation models, and builds upon existing in-situ measurements and modeling programs funded by various state and federal agencies. In addition, COMPS links to the USF Remote Sensing Laboratory. This observing system fulfills all of the requirements of the Coastal Module of the Global Ocean Observing System (CMGOOS). Data and model products are disseminated in real-time to federal, state, and local emergency management officials *via* the internet (URL http://comps.marine.usf.edu/). Data collected include current speed at several depths and several locations. Data products include current vectors for the West Florida Shelf and these are updated daily. These products have already been analyzed by COMPS personnel, and will be used to infer water movement at the time of dispersal.

4 Methods

4.1 Offshore Field Sampling Procedures

Release of fresh, nutrient rich water from the dispersal may result in a surface lens of reduced salinity, nutrient rich water that has the potential to enhance the growth of phytoplankton and induce Harmful Algal Blooms (HABs). FDEP will use a variety of sensors to detect the low or reduced salinity water and/or increased phytoplankton biomass (as *in vivo* fluorescence). Typical salinities for the region where dispersal will take place are approximately 36 psu. FDEP expects that dispersal of the water will reduce surface sea water salinity to be detectable by our instruments.

This project is not a hypothesis driven experiment. It is designed to detect the release of low salinity, nutrient enriched water into a high salinity, oligotrophic oceanic environment, and to collect samples to determine if the release of this water is accompanied by growth of local phytoplankton populations and HABs.

Monitoring for detection of changes in the physical and chemical characteristics of the water column and the potential development of HABs within the dispersal area will be conducted using a combination of instrument packages and water column samples. Two sensor packages will be used: one equipped with an *in situ* instrument package deployed from the monitoring vessel. The *in situ* sensor package includes the following instruments (see Table 1): a chlorophyll fluorometer, a CTD, and a transmissometer (which measures light transmission).

Table 1. Instruments deployed for the offshore plume sampling. [1]

Parameter	Lab	Units	Instrument
Conductivity	USF	mS/cm	SeaBird TSG
Temperature	USF	°C	SeaBird TSG
Pressure	USF	M	SBE 25 CTD
Transmissometry	USF	m^{-1}	WET Labs 25-cm (660-nm)
Bottom depth	USF	M	Unknown
Navigational position	USF	degrees	Northstar 941x
Sigma-T	USF	No units	SeaBird TSG
Chlorophyll	USF	Mg/L	WET Labs WETStar fluorometer

This *in situ* instrument package will be deployed off the monitoring vessel. A winch and vessel speed will be used to control the depth of the towed sensor package. The sensor package can be raised or lowered using the winch at a rate of 0 to 1.0 m/sec.

Depending on the vessel's speed and winch operation, the *in situ* instrument package can operate in two different modes: vertical profile, or constant-depth towing. In vertical profiling mode, data is acquired as a function of depth while the vessel remains stationary. During constant depth towing mode, the *in situ* instrument package is towed at a constant depth while the vessel is underway. The HAB development monitoring may utilize the *in situ* instrument package in either mode on each cruise survey at selected survey stations and also underway. These data provide general oceanographic (physical and biological) conditions in three dimensions, and therefore can and will be used to study where the dispersed water will go (satellites only detects the surface water) and how the conditions may be changed by the dispersal (*e.g.*, does the dispersal stimulate a bottom rather than surface bloom?).

A second package consisting of a CTD, chlorophyll fluorometer, transmissometer, and particle scattering meter is housed on deck in a tank through which sea water from a

[1] exact instrument configuration may vary depending on availability

constant depth (2m) is circulated. Both packages combine navigation data with the instrument output so that maps can be drawn from the data. The towed package will provide a three-dimensional view while the on deck package will provide a near surface map. A third set of samples will be obtained using the ships CTD –rosette system. Vertical profiles to 50m or to the depth of the mixed layer which ever is greater, will be taken at stations noted in Fig. 1. Water samples will be takes at the surface and three other depths that correspond to changes in water column density. A maximum of surface and three other depths will be sampled. Mixing of the dispersed water to the depth of the mixed layer could occur given favorable wind conditions. Therefore, it is necessary to sample the subsurface layers to determine if shifts in biomass and/or species composition have occurred that might later be related to nutrient enrichment from the discharged water.

Sample Storage Conditions.

Discrete chlorophyll samples collected aboard the monitoring vessel and dispersal vessel will be stored at -20º C (chlorophyll samples may be filtered), or analyzed on-board. Additional sample storage conditions are presented in Table 2.

Table 2. Analyte, Sampling Method, Volume, Preservation, and Holding Times.

Analyte (Analytical Laboratory)[a]	Matrix	Method	Container Type	Preservation	Holding Time
		Laboratory Analyses			
Chlorophyll (USF)	Water	Exctraction and laboratory spectrophotometer	500 mL polyethylene	Filter and store at –20° C]	6 hours [48 hours]
		Field Analyses			
Temperature	Water	SeaBird TSG	NA	NA	*In situ*
Pressure (depth)	Water	SBE 25 CTD	NA	NA	*In situ*
Transmissometry/ Turbidity	Water	WET Labs 25cm (660-nm) transmissometer	NA	NA	*In situ*
Conductivity	Water	SeaBird TSG	NA	NA	*In situ*
Chlorophyll	Water	WET Labs WETStar fluorometer	500 mL polyethylene	NA	*In situ*
(a) USF: University of South Florida – College of Marine Science NA = Not applicable.					

4.2 Harmful Algal Bloom (HAB) Development Methodologies and Interpretation

Live and preserved (Logul's iodine) samples will be collected for the Florida Fish and Wildlife Commission, Florida Marine Research Institute (FMRI).

Sampling and analyses (identification/counts) of phytoplankton will be performed by FMRI following the methods described below. Bloom conditions will be defined based upon existing protocols developed by FMRI (bloom initiation indicated at cell numbers > 1000 L^{-1}).

FMRI Counting Method for Preserved Samples:

1. Mix sample thoroughly.
2. Pour up to 25mL in a standard Utermöhl chamber.
3. Settle for 24 hours.
4. Examine 40 fields* at high magnification (*i.e.*, 40x), identify and count species. (This is to identify small and/or numerous species within the sample.)
5. Examine entire chamber at low magnification (*i.e.*, 10x), identify and count species. (This is to identify large and/or uncommon species within the sample.)

*Fields are chosen either in a predetermined pattern (*i.e.*, widest transects longitudinally and laterally) or using a randomization chart of coordinates.

These methods are adapted from Hasle (1978) and Venrick (1978), both in Sournia, A. (ed.). 1978. Phytoplankton Manual. UNESCO, Paris.

FDEP will utilize the data provided from these remote sensing, real-time monitoring, and modeling capabilities (in conjunction with *in situ* monitoring), in order to effectively manage the dispersal of treated wastewater through observation of existing oceanographic conditions.

If elevated chlorophyll and toxic phytoplankton species are found in samples collected within the region of dispersal, FDEP would stop or reduce dumping until previous dispersals have either mixed and/or been transported out of the area and chlorophyll concentrations and HAB abundance have returned to below bloom levels.

This will be achieved in several steps:

1) Does remote sensing detect any anomaly event associated with the discharge? Here "anomaly" means contrast with both historical data at the same dispersal location (IMaRS has daily data back to 1994 for temperature and 1997 for ocean color) and concurrent data in the adjacent water. If yes, a cruise survey will be scheduled immediately to investigate the anomaly feature in the field, in particular, is it red tide? If yes, the dispersal plan needs to be revised.

2) In case of cloud cover when remote sensing doesn't work, cruise surveys will be used to search for any anomaly features in the discharge area. Previously mentioned management practice will be used.
3) Routine cruise surveys, to obtain 3-D view of the dispersal area, as well as to provide ground truth data for remote sensing, will be performed.
4) Remote sensing and COMPS data will be used to infer the movement of the dispersed water. Because the dispersal area is within the large loop current, it is very likely to be carried by the current to the Gulf Stream. IMaRS will monitor any anomaly event along this whole path.
5) How can we be certain that a red-tide event, once identified, is related to this discharge? IMaRS will study the location and duration of the event, as referenced to the dispersal. If it occurs at the same dispersal location within 1-2 days but did not exist before the dispersal, we are certain that they are related. Scientifically speaking, no one knows HOW a red tide bloom starts – this is still a hot research topic. However, we do know that red tide favors a certain number of oceanographic conditions, for example, ambient nutrient, moderate temperature, and sunlight protection (from dissolved matter or other species).

HABs of *Karenia brevis* on the West Florida Shelf are not typically found in waters >100nm offshore (Steidinger, 1986). Conditions in this region of the Gulf of Mexico are typically oligotrophic with high salinity (36 psu), low nutrient (<0.5 uM nitrate, nitrite and ammonia; approx. 0.2 uM phosphate), and low chlorophyll concentrations in surface water (<0.5 ug/l) unless there is a bloom of the cyanobacterium *Trichodesmium* spp. The dispersed water, which is fresh with high nutrients, may, if not effectively mixed during discharge, form a surface lens due to its lower density relative to sea water. Given the high nutrient concentrations in the dispersed water, phytoplankton growth can be expected in near surface waters and/or within the surface mixed layer. Vertical CTD profiles will give a 3-D picture of the dispersal area for physical, chemical, and biological characteristics. Development of phytoplankton blooms can then be related to the characteristics indicative of dispersal water as discussed previously.